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Quantitative Assessment of the Adsorptive Behavior of Viruses to Soils

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16. ABSTRACT

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Quantitative Assessment of the Adsorptive Behavior of Viruses to Soils

Charles P. Gerba[†] and Sagar M. Goyal*

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030

Irina Cech and Gregory F. Bogdan

School of Public Health, University of Texas, Houston, Texas 77030

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■ To evaluate the potential for groundwater contamination by viruses, it is essential to understand the processes controlling virus adsorption to soil. Recent laboratory studies have indicated that the degree of viral adsorption is highly type and strain dependent. The purpose of this study was to statistically reexamine earlier data by grouping studied variables into a number of broad categories. The results indicated that different types and strains of viruses can be grouped by their ability to be similarly affected by certain soil characteristics. Viruses could be grouped into two general categories. For group I, the most important factors affecting adsorption were pH, organic matter, and exchangeable iron content of the soil. No studied soil characteristic was found to be significantly associated with adsorption of group II viruses. The implication is that adsorption–elution of group I viruses is more sensitive to certain soil characteristics than that of group II. Certain types of coliphages were found to be better models for some types of enteroviruses than others.

Almost half of all documented waterborne disease outbreaks in the United States result from contaminated groundwater (*1*). An understanding of the factors that control virus migration through subsurface soil systems is critical in the management of septic tank and wastewater land treatment systems. Virus removal by soil is believed to be largely caused by adsorption, which is controlled by a number of factors, including composition of the soil, ionic strength, pH, flow rate, etc. (*2*). We have recently reported on the adsorption of 28 different animal and bacterial viruses to nine different soil types using a batch adsorption procedure (*3*). Poliovirus adsorbed well to the majority of the test soils, but some coxsackieviruses and echoviruses demonstrated varied adsorption patterns, depending on the origin of the virus. Reference strains of coxsackievirus (B1 through B6) were found to adsorb readily to soils, whereas natural isolates of coxsackievirus B4 adsorbed poorly. A great deal of variability was also observed between adsorption of different strains of echovirus 1, indicating that virus adsorption to soils is highly strain dependent. The adsorption of coliphages also varied greatly. Landry et al. (*4*) recently studied the elution and adsorption of both field and reference strains of enteroviruses to soil cores. The elution of the viruses by rainwater appeared to be strain dependent, indicating a strain or type variation in the overall charge characteristics of each virus. Earlier studies conducted by our laboratory on virus adsorption failed to reveal a consistent predictive pattern of adsorption as related to any particular soil property (*3*). The purpose of the current study was to reexamine earlier data statistically by grouping the multiplicity of studied variables into a smaller number of broader categories, thus simplifying the task of data interpretation.

Materials and Methods

Soils. Nine different soils from different sources were used. The major physicochemical properties of these soils have previously been described in detail (*5*).

Viruses. Poliovirus 1 (strain LSc), echovirus 1 (Farouk), echovirus 7 (Wallace), and coxsackievirus B3 (Nancy) were obtained from the Research Resources Branch, National Institutes of Health, Bethesda, MD. Echovirus 1 isolates V212, V239, and V248 and coxsackievirus B4 isolates V216 and V240 were isolated from groundwater beneath a wastewater disposal site. These viruses were identified by the use of combination antiserum pools as described by Melnick et al. (*6*). Simian rotavirus SA11 was obtained from H. H. Malherbe (*7*). Five different *Escherichia coli* bacteriophages—MS-2, ϕX174, T2, T4, and f2—were obtained and grown as previously described (*8*).

Adsorption Studies. Adsorption of virus to soil was determined by addition of 2 g of test soil to a 2-mL suspension of virus containing 10^6–10^7 plaque-forming units in one of the solutions in a test tube. The test tube was stoppered, hand shaken, and then placed on a rotary shaker at 200 rpm for 30 min. The soil was then removed from suspension by centrifugation for 4 min at 2500 g, and the supernatant fluid was assayed. A control suspension of virus without soil was treated in the same manner. The difference in titer between the control and the sample containing soil was used to determine the quantity of virus adsorbed to soil. The 30-min contact time was selected after time-rate tests indicated that, for all practical purposes, equilibrium was attained during that period. All experiments were performed with deionized water. The results reported are the average of two to five experiments.

Statistical Analysis. Data analysis was done in the following sequence. First, the differences in virus adsorption in nine tested soils were examined by the analysis of variance. Details of this type of analysis can be found in ref *9*. Second, the assayed virus types were grouped on the basis of their amount of adsorption by using factor analysis. Third, mean adsorptions for similarly behaving virus groups were calculated and regression analyses of those means on soil characteristics were performed in an effort to determine which of these characteristics are potentially useful as predictors of virus adsorption.

Results

Table I presents the results of a two-way ANOVA (analysis of variance) for 14 virus types and 9 soil types. As follows from this table, significant differences in adsorption were observed both from virus to virus and between soils.

Figure 1 shows the results of factor analysis. Visual inspection of the factor plot suggested that viruses apparently can be grouped, on the basis of their adsorption, into two general categories, i.e., higher and lower adsorption to soils (Table II). The exception appears to be bacteriophage f2, which occupies an intermediate position between two groups.

Table III provides descriptive statistics for each virus type and for virus groups I and II. The means of adsorption of those two groups are statistically significantly different. Bacteriophage f2 is probably a special case, as its mean adsorption of 16.4% was significantly lower than that of either of the other groups (Table III).

To evaluate the effect of specific characteristics of various soils on virus adsorption, we subdivided these characteristics

[†] Department of Microbiology, University of Arizona, Tucson, AZ 85721.

Table I. Two-Way Analysis of Variance of Adsorption for 14 Virus Types and 9 Soil Types

source of variation	sum of squares	degrees of freedom	mean square	F ratio	significance of F ratio
within + residual	66 455	104	6389		
soil	54 851	8	6856	11	0.000 01
virus	50 581	13	3891	6	0.000 01

Table II. Virus Grouping by Adsorptive Behavior

group I		group II		group III?	
members	code no.	members	code no.	member	code no.
coxsackie B4 (V216)	8	polio 1 (LSc)	1	f2	14
coxsackie B4 (V240)	9	echo 7 (Wallace)	3		
echo 1 (Farouk)	2	coxsackie B3 (Nancy)	4		
echo 1 (V212)	5	T4	12		
echo 1 (V239)	6	T2	11		
echo 1 (V248)	7				
ϕX174	13				
MS-2	10				

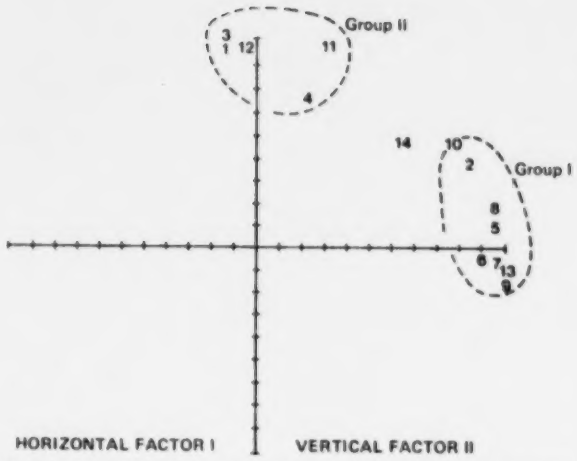

Figure 1. Factor analysis of virus adsorption to nine soil types. The axes on this graph show the scores, from 0 to 1, of virus similarities in terms of their mean adsorption. Viruses appear to cluster in two distinct groups (as shown by dotted lines). Bacteriophage f2 is apparently an exception, as its mean adsorption is markedly different from those of the two major groups. In the statistical analyses, the f2 virus is regarded as a special group. Numbers in the figure refer to the codes given in Table II.

into three sets. The first set contained variables describing general physical and chemical characteristics of the soils. Included in this set were pH, percent organic matter, conductivity, cation exchange capacity, and soil surface area. The second and third sets contained variables describing specific chemical elements in the soils (i.e., set 2—concentration of magnesium, calcium, phosphorus, aluminum, and iron; set 3—concentration of exchangeable magnesium, calcium, phosphorus, aluminum, and iron). The adsorption (y), computed as an average adsorption of all viruses in a given group, was regarded as a function of the various characteristics (denoted by $x_1, x_2, \ldots, x_5$) of the soil group. The hypothesized functional dependence

$$y = f(x_1, x_2, \ldots, x_5)$$

was studied by stepwise multiple regression using the SPSS ("Statistical Package for the Social Sciences") computer program, Version 7 (*10*).

The results of the regression analyses are summarized in Tables IV–VI. These must be regarded in the proper perspective and interpreted with certain caution. Because of experimental limitations, the degree of freedom in the regression analyses is small, and therefore the reliability of the

Table III. Descriptive Statistical Summary of Virus Adsorption to 9 Soil Types, by Individual Virus Types and by Their Groups [a]

virus	adsorption, %			
	mean	SD	minimum	maximum
echovirus 1 (Farouk)	39.19	35.00	11.00	99.70
echovirus 1 (V212)	45.08	31.80	1.00	99.70
echovirus 1 (V239)	46.89	29.98	5.00	98.00
echovirus 1 (V248)	45.98	37.62	0.10	99.90
coxsackievirus B4 (V216)	38.14	29.66	2.00	99.30
coxsackievirus B4 (V240)	55.76	36.36	0.10	98.70
MS-2	45.34	26.58	16.60	99.91
ϕX174	34.51	44.91	0.01	99.98
group I	43.86	33.25	0.01	99.98
poliovirus 1 (LSc)	85.26	21.60	42.00	99.90
echovirus 7 (Wallace)	68.13	40.06	8.00	99.90
coxsackievirus B3 (Nancy)	81.07	22.48	35.00	99.61
T2	72.12	31.69	8.40	99.99
T4	83.12	32.52	0.01	99.70
group II	77.94	29.88	0.01	99.99
f2				
group III	16.44	32.39	0.01	99.20
all viruses	56.97		0.01	99.99

[a] As followed from the factorial analysis.

regression results is limited. The results are interesting, however, for the development of hypotheses for future experiments. They suggest that particular soil characteristics do influence adsorption to a different degree, depending on the virus group. For example, in virus group I, of all the soil variables studied, the most important appeared to be pH. This single factor, which was found to be inversely correlated with adsorption, explained statistically ~83% of the variation in mean adsorption of viruses in group I (Table IV). Another variable of importance to group I from the same set of soil characteristics appears to be content of organic matter. For this variable, the direction of association was also inverse. From other sets of soil variables, the only element found to be statistically significant for virus group I was exchangeable iron (Table IV). The direction of association for this variable was positive.

The regression analysis of adsorption on soil characteristics for viruses in group II showed that none of the characteristics was a significant predictor of viral adsorption (Table V).

Inasmuch as type f2 was not included in either virus group I or II, it was analyzed by itself (Table VI). For this virus, the concentration in soil of exchangeable aluminum was found to be a significant predictor of f2 adsorption. The direction of this association was positive.

Discussion

To evaluate the potential for groundwater contamination by viruses, it is essential to understand the processes controlling virus adsorption to soil. Recent laboratory studies have indicated that the degree of viral adsorption is highly type and strain dependent (*3*). In a previous study by this laboratory, one-to-one linear correlation between soil characteristics and virus adsorption failed to reveal any one single soil property that was directly influencing virus adsorption (*3*). Some soil properties were found to be associated with the degree of adsorption of some types of viruses but not with others, and thus no consistent pattern was detected. In the course of the present study, it became apparent that in the

Table IV. Stepwise Multiple Regression of Mean Virus Adsorption in Group I on Soil Characteristics

dependent variable	significance of individual variables	multiple R	R^2	R^2 change	simple R	overall F	significance of regression
first set of soil variables:							
pH	0.004	0.911	0.8300	0.8210	−0.9110	24.404	0.004
organics	0.032	0.976	0.9525	0.1226	−0.4920	40.132	0.002
ion exchange capacity	0.094	0.991	0.9839	0.0314	−0.0435	61.259	0.003
surface area	0.149	0.998	0.9955	0.0116	−0.1130	112.196	0.009
conductivity	0.754	0.998	0.9962	0.0006	−0.2662	52.332	0.105
second set of soil variables:							
total phosphorus	0.138	0.619	0.3830	0.3830	−0.6189	3.100	0.138
total calcium	0.672	0.643	0.4135	0.0305	−0.4581	1.400	0.344
total aluminum	0.834	0.650	0.4234	0.0010	−0.3377	0.730	0.597
total iron	0.764	0.675	0.4555	0.0321	−0.2024	0.420	0.793
third set of soil variables:							
exchangeable aluminum	0.056	0.743	0.5515	0.5515	0.7427	6.150	0.056
exchangeable iron	0.025	0.943	0.8902	0.3386	0.5755	16.216	0.012
exchangeable magnesium	0.359	0.960	0.9209	0.0307	−0.1645	11.649	0.037
exchangeable calcium	0.347	0.977	0.9546	0.0337	−0.1608	10.523	0.089
exchangeable phosphorus	0.835	0.978	0.9576	0.0029	−0.4409	4.517	0.342

Table V. Stepwise Multiple Regression of Mean Virus Adsorption in Group II on Soil Characteristics

dependent variable	significance of individual variables	multiple R	R^2	R^2 change	simple R	overall F	significance of regression
first set of variables:							
ion exchange capacity	0.106	0.661	0.437	0.437	0.661	3.887	0.106
conductivity	0.214	0.797	0.636	0.198	0.552	3.493	0.133
surface area	0.064	0.950	0.903	0.267	0.592	9.333	0.050
organic matter	0.485	0.964	0.929	0.026	0.386	6.532	0.137
pH	0.413	0.987	0.974	0.045	−0.044	7.509	0.270
second set of variables:							
total magnesium	0.133	0.625	0.391	0.391	0.625	3.213	0.133
total calcium	0.146	0.814	0.663	0.272	0.373	3.943	0.113
total phosphorus	0.293	0.884	0.781	0.118	0.250	3.573	0.162
total iron	0.185	0.962	0.927	0.145	0.602	6.324	0.141
total aluminum	0.824	0.965	0.932	0.005	0.617	2.749	0.427
third set of variables:							
exchangeable magnesium	0.126	0.634	0.402	0.402	0.634	3.365	0.126
exchangeable aluminum	0.196	0.792	0.627	0.224	0.365	3.357	0.139
exchangeable calcium	0.316	0.865	0.748	0.121	0.517	2.967	0.198
exchangeable phosphorus	0.597	0.888	0.789	0.041	0.518	1.869	0.378
exchangeable iron	0.683	0.915	0.837	0.048	0.238	1.028	0.631

Table VI. Stepwise Multiple Regression of f2 Virus Adsorption on Soil Characteristics

dependent variable	significance of individual variables	multiple R	R^2	R^2 change	simple R	overall F	significance of regression
first set of variables:							
pH	0.215	0.535	0.287	0.287	−0.535	2.009	0.215
conductivity	0.729	0.557	0.310	0.024	−0.091	0.900	0.476
exchange capacity	0.896	0.561	0.315	0.005	0.216	0.460	0.730
surface area	0.567	0.666	0.443	0.128	0.116	0.398	0.803
organic matter	0.893	0.678	0.459	0.016	−0.044	0.170	0.940
second set of variables:							
total phosphorus	0.262	0.492	0.242	0.242	−0.491	1.594	0.262
total aluminum	0.418	0.608	0.370	0.128	−0.085	1.175	0.397
total iron	0.209	0.812	0.659	0.289	−0.140	1.934	0.301
total magnesium	0.658	0.836	0.699	0.040	−0.157	1.162	0.511
total calcium	0.196	0.986	0.972	0.273	−0.188	7.055	0.278
third set of variables:							
exchangeable aluminum	0.001	0.957	0.916	0.916	0.957	54.602	0.001
exchangeable iron	0.103	0.980	0.960	0.044	−0.218	48.289	0.002
exchangeable phosphorus	0.136	0.991	0.983	0.023	−0.352	58.452	0.004
exchangeable calcium	0.511	0.993	0.987	0.004	−0.250	38.578	0.025

search for such a pattern it might be helpful to group factors influencing virus adsorption as well as viruses themselves with respect to their adsorptive properties.

The results reported here indicate that different types and strains of viruses can, indeed, be grouped by their ability to be similarly affected by certain soil characteristics. Viruses were grouped into two general categories with mean adsorption of 44% for virus group I and 78% for virus group II. Bacteriophage f2, because of its 16% adsorption being entirely outside of the above-indicated range, was placed in a third category.

For virus group I, the most important factors appeared to be pH, content of organic matter, and exchangeable iron. None of the studied soil characteristics was found to be significantly associated with adsorption of viruses in group II.

This does not mean that the adsorption of viruses in group II is not affected by the same factors, but that over the range of values of these factors in the soils studied there was no obvious relationship. The implication is that adsorption–elution of group I viruses is more sensitive to changes in pH, organics, and exchangeable iron over the ranges studied. This was borne out in a previous study on the adsorption of these same viruses to marine sediments (*11*). In that study the adsorption of echovirus 1 (strain Farouk and isolate V239) and coxsackievirus B4 (isolate V240) to sediment was influenced by the salinity, pH, and humic acid content of the seawater in which the adsorption–elution experiments were conducted. Under the same conditions poliovirus 1 (strain LSc), echovirus 1 (strain Wallace), and coxsackievirus B3 (strain Nancy) adsorptive behavior was not affected.

Group II viruses adsorbed very well to most soils, sludges, and marine sediments, while group I viruses adsorbed to a lesser degree and were affected more by pH and the organic content of the environment (*3*, *11*, *12*). Coliphage f2 may represent an intermediate in behavior between the major groups; since overall it adsorbed the least to all of the soils, it may actually represent a third group.

Differences in adsorption between the different types and strains of viruses probably resulted from variability in the configuration of proteins on the outer capsid of the virus, since this could influence the net charge on the virus (*2*). The net charge on the virus would affect the electrostatic potential between virus and soil, and thereby could influence the degree of interaction between the two particles. It is possible that the net charge on group I viruses was more easily influenced over the range of pH values and organic matter concentrations found in nature than group II viruses. It would appear that groundwater would be more susceptible to contamination by viruses with properties similar to those of group I. This is not to say that group II virus adsorption is not influenced by organics, pH, and salt concentration, as is already known (*2*, *13*), but that over the range of soil properties used there was no observable predictive relationship.

Although virus strains of the same type were grouped together because of similar adsorptive behavior, this may not have necessarily held true if additional strains of viruses had been studied. The results also indicate that, while adsorption of different strains of the same virus type may be influenced by the same factors, the degree to which they are influenced is strain dependent.

The different bacteriophages also could be grouped according to their adsorptive behavior. It would appear that coliphages MS-2 and ϕX174 could be used as potential models for studying the adsorptive behavior of group I animal viruses while T2 and T4 could be used as models for group II animal viruses. Similarities in the adsorption of poliovirus 1 and coliphage T2 to soils and clay have been observed previously (*13*, *14*). Differences in the adsorptive behavior of poliovirus 1 and f2 and of f2 and T2 to soils have also been previously reported (*15*, *16*).

The adsorption of group I viruses was affected by the amount of organic matter for the soils studied, even though the amount of organic matter was quite low (0.27–4.2%). Adsorption of coliphage ϕX174, which was placed in group I, was found by Burge and Enkiri (*17*) to be statistically significant for association with organic content of the soils. The soil pH was found by these investigators to be the most significant factor controlling the rate of ϕX174 adsorption to soil.

Cation exchange capacity and specific surface area were also related to adsorption, but to a lesser degree. Drewry and Eliassen (*18*) reported that, while clay content and ion exchange capacity influenced the adsorption of T2, T4, and f2, these soil properties could not be used to predict virus adsorption.

The results of this study may also be useful in the development of methods for virus concentration from water which are dependent on adsorption. Recently, Morris and Waite (*19*)

reported on the efficiency of concentration of viruses by the commonly used beef extract organic flocculation method, which depends on virus adsorption–elution from an organic floc. They reported large differences in the efficiency of this method for the concentration of different enteroviruses. The efficiency of concentration was as follows: coxsackievirus B4, 9%; echovirus 1, 7%; coxsackievirus B3, 98%; and poliovirus 1, 40%. Bitton et al. (*20*), using nonfat dry milk as an organic flocculant for virus reconcentration, also observed that the efficiency of concentration of poliovirus 1 and coxsackievirus B3 was almost 10-fold greater than that of echovirus 1. These results appear to confirm our data and indicate major differences in the adsorptive behavior of group I viruses (coxsackievirus B4, echovirus 1) and group II viruses (poliovirus 1 and coxsackievirus B3).

In summary, it would appear that the adsorption of certain types and strains of viruses is more susceptible to the influence of soil pH, organic matter, and exchangeable iron and aluminum than that of others. Certain types of coliphages may be better models for some types of enteroviruses than others. These differences should be taken into consideration in both field and laboratory studies on virus adsorption and migration through soil.

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